J. HYM. RES.
Vol. 3, 1994, pp. 107-117

Variation in the Haemolymph Protein Composition of Confined *Apis Mellifera* and Partial Restoration of Vitellogenin Titre by Juvenile Hormone Analogue Treatment

M. M. G. Bitondi, Z. L. P. Simões, A. M. do Nascimento and S. L. Garcia

Dept. Biology, Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto Universidade de São Paulo, C.E.P. 14040-901 Ribeirão Preto, S.P., Brasil (AMN and SLG are undergraduate fellows of the Convênio CNPq/USP)

Abstract.—Haemolymph proteins, especially vitellogenin (Vg), were investigated in confined *Apis mellifera* workers, that were fed different diets and treated with juvenile hormone (JH) I, III or with pyriproxifen (PPN). Vg and total protein concentrations were drastically decreased in the haemolymph of workers removed from the colony and confined for different periods of time. SDS-PAGE analysis demonstrated that confinement also caused induction and repression of the synthesis of certain haemolymph proteins. All of these changes occurred even when the confined workers were fed a protein-rich diet. In workers with Vg deficiency induced by confinement PPN, but not JHI or JHIII, induced a partial increase in Vg concentration.

INTRODUCTION

The vitellogenin (Vg) of *Apis mellifera*, the precursor of vitellin —the major protein of yolk— is a glycolipoprotein produced in the fat body (Harnish and White 1982; Trenczek and Engels 1986; Shapiro et al. 1988; Wheeler and Kawooya 1990). In most insects, Vg synthesis may be controlled by the diet, which obviously provides the materials and energy needed for this process (Bianchi and Pereira 1987; Bownes 1989; Bownes and Reid 1990). However, other factors in addition to diet are also involved. Many studies have been conducted on the role of neurosecretory cells (Elliott and Gillott 1978) and neurohormones (Keeley and Mckercher 1985; Keeley *et al.* 1988; Girardie *et al.* 1992), and of juvenile hormones and ecdysteroids in the regulation of Vg synthesis (Adams et al. 1985; Borowsky *et al.* 1985; Hagedorn 1985; Schwartz *et al.* 1985; Wojchowsky and Kunkel 1987; Adams and Filipi 1988; Keeley *et al.* 1988; Ma et al. 1988; Röseler and Röseler 1988; Wyatt 1988; Bownes 1989; Bownes and Reid 1990; Davis *et al.* 1990; Hatakeyama and Oishi 1990; Yin *et al.* 1990; Agui *et al.* 1985, 1991; Don-Wheeler and Engelman 1991; Socha *et al.* 1991).

In *Apis mellifera*, a highly eusocial insect, the control exerted by the queen over the workers represents an additional factor influencing Vg synthesis. A queen pheromone inhibits oocyte development in the workers. As a result Vg is not incorporated into the oocytes, although Vg is detected in the workers haemolymph. However, in queenless colonies the Vg titre of workers increases, reaching a level similar to that observed in the queens, followed by oocyte growth and oviposition (Engels 1974; Engels and Fahrenhorst 1974).

How Vg synthesis is regulated in *A. mellifera* still remains an interesting question, and aspects of this regulation have been studied in queens, workers and drones. In queens Vg synthesis does not depend on functional *corpora allata* and is not mediated by JH (Engels and Ramamurty 1976; Kaatz 1985). Similarly, JH topical application does not increase Vg synthesis in drones (Trenczek *et al.* 1989). But in workers, Rutz *et al.* (1976) and Fluri *et al.* (1977) observed that low JH doses applied on 6 day old workers stimulate Vg synthesis whereas high doses have an inhibitory effect. Furthermore, Rutz *et al.* (1976) observed a correlation *in vivo* between low JH titre and Vg synthesis. Within the first few days after worker emergence, characterized by low JH titres in haemolymph there is an increase in Vg synthesis. After this period Vg

synthesis decreases while JH titre increases. This increase in JH titre was confirmed by Huang *et al.* (1991) and the temporal changes in Vg titre observed by Rutz *et al.* (1976) were similar to those related by Engels *et al.* (1990).

The investigation of factors that affect *A. mellifera* Vg synthesis can help to understand how this protein is regulated. In the present work, the effect of some factors such as changes in social environment (confinement of workers with or without a queen) and diet (protein-rich or not) and JH or PPN (pyriproxyfen, 2-[1-methyl-2(4-phenoxyphenoxy) ethoxyl] pyridine, a JH analogue, were investigated not only on worker Vg synthesis but also on other haemolymph proteins.

MATERIALS AND METHODS

Apis mellifera

We used "wild type" Africanized *Apis mellifera* bees (hybrids of European *A. m. ligustica*, *A. m. carnica*, *A. m. mellifera* and the African bee *A. m. scutellata*) from the Experimental Apiary of the Department of Genetics, Faculty of Medicine of Ribeirão Preto, University of São Paulo.

Combs containing workers ready to emerge were removed from colonies and placed in an incubator whose temperature (34°C) and R.H. (80%) were similar to those in the colony. The workers that emerged within 15-20 hours were collected. About 100 newly emerged workers were marked on the thorax and put back into a small colony, formed by a queen, approximately 3000 workers (hive and forager bees), brood (eggs, larvae and pupae) and, sometimes, a few drones. Presence of nectar and pollen into the combs were also checked.

Confinement

The remaining workers were separated into groups of 150-200 and immediately submitted to confinement in 8 x 11 x 13 cm wooden cages with a sliding glass door and a screened bottom. The workers in these cages were placed in an incubator at 34°C and 80% R.H. and confined for 6 days (short confinement) or 15-16 days (long confinement). The confined bees received water and food *ad libitum*. The diet consisted of 50% sugar in water (syrup), a mixture of pollen from the comb (beebread) and candy (powdered sugar and honey), or only candy.

In three experiments, three naturally mated queens aged 60-90 days were removed from the respective colonies and confined with groups of 150-200 newly emerged workers. These groups formed by workers and a queen were confined for 15-16 days, and fed on the mixture of beebread and candy. Water was also supplied.

Treatment of confined workers with JHIII, JHI and PPN

Some groups of 150-200 workers confined for 6 days were treated with JHIII, JHI or PPN applied topically to the abdominal cuticle. Each worker received 1 µl of a hormone solution in acetone at a concentration of 1 µg/µl, administered in two equal doses, the first immediately after emergence, before the introduction into the cages, and the other on the third day after rapid anesthesia with gaseous nitrogen. Two worker groups were treated with JHIII, two groups with JHI and nine groups with PPN.

Three control groups were prepared in parallel: the first consisted of marked workers reintroduced into the colony (control a), the second formed by confined workers treated with two 1 µl doses of acetone on the first and third days, respectively (control b). This group was also submitted to a rapid anesthesia with gaseous nitrogen at third day, immediately before acetone treatment. The third group consisted of untreated confined workers (control c). All worker groups, except control a, were allowed to feed *ad libitum* on the mixture of beebread and candy. Water was also supplied.

Haemolymph

For collecting haemolymph the workers were anesthetized with gaseous nitrogen and immobilized on dissection plates. Haemolymph was extracted through a small superficial incision in the dorsal cuticle between the 2nd and 3rd tergites.

Haemolymph was withdrawn from 6 day old and 15-16 day old confined workers that were fed on different diets, treated or not with hormones or acetone and mantained with or without a queen (Table 1). Haemolymph was also extracted from colony reared workers (6 or 15-16 day old) and from newly emerged workers before confining or

returning them to the colony.

Haemolymph pools were prepared from groups of at least 80 workers obtained from the same confinement cage. Similar pools were prepared with the haemolymph of at least 20 workers of the same age maintained in the colony and of 12 newly emerged workers. Phenylthiourea was added to the pools. Haemolymph pools were centrifuged at 3080 g for 10 min at 0°C and the supernatant was stored at -20°C.

Rocket immunoelectrophoresis

Immunoelectrophoresis was used for the quantitative determination of the vitellogenin fraction in haemolymph. Monospecific vitellogenin antiserum produced in rabbits (Simões 1980) was added at a 1% concentration to 1% agarose gels prepared with 0.06 M Tris-HCl buffer, pH 8.6. Immunoelectrophoresis was carried out at 10°C for 16 hours, at 0.08 V/cm gel. The same buffer used in the gel was used in the electrode compartments at a concentration of 0.3 M. The gels were stained with Coomassie Blue R-250. The height of the peaks detected (reported as mm) was proportional to the amount of antigen. The values obtained for confined workers were compared to those for workers of the same age maintained in colonies. The Vg peaks detected in colony reared workers (control c) was considered to be 100%.

Total protein

Protein concentration in the haemolymph pools was determined using bovine serum albumin as a standard (Bradford 1976).

SDS-PAGE

Soluble haemolymph proteins were separated by SDS-PAGE according to the method of Laemmli (1970) except that SDS was not used in the separating and stacking buffers. A 5-12% acrylamide gradient was used on a 0.7-mm thick gel. Electrophoresis was run at 12mA constant current at 10°C until bromophenol blue tracking dye reached the bottom of the slab.

Haemolymph samples (5 µl) from confined and newly emerged workers (diluted 1: 2, v/v, in sample buffer) and from workers maintained in the colonies (diluted 1: 20 or 1: 40, v/v) were applied to the acrylamide gel.

RESULTS

Confinement blocks the increase of haemolymph Vg

The confinement of workers for a period of 6 days after emergence prevented the increase of Vg titre in haemolymph that normally occurs in workers in colonies. This occurred even when beebread, the natural source of bee protein was supplied to the confined bees. In some of the haemolymph pools an extremely low Vg titre was detected (Fig. 1, wells 5 and 6), but, on average, Vg titres in these pools corresponded to 6.0 ± 2.1% (Table II) of that present in workers maintained in the colonies under natural conditions (Fig. 1, wells 1 and 2).

A small increase in Vg titre was observed in 15-16 day confined workers (Fig. 1, wells 3 and 4). This only occurred when the workers were fed beebread and candy. Even with this diet, the Vg concentration was much lower than in workers of the same age maintained in the colonies (Fig. 1, wells 1 and 2).

Confinement changes the protein pattern of haemolymph obtained by SDS-PAGE

The pattern of soluble haemolymph proteins from six day confined workers (Fig. 2, lane 4) differed from that observed in workers maintained in colonies during the same period of time (Fig. 2, lane 3). In the confined bees, in addition to the fact that the Vg band (as determined by Trenczek *et al.* 1989) was very weak, the *a* and *c* polypeptides were not observed, whereas the *b* polypeptide formed a strong band. This polypeptide corresponded to a weak band in the workers living in colonies. These were the most obvious differences, however differences between low molecular weight polypeptides, were also observed.

In Fig. 2, lanes 1, 2 correspond to the haemolymph protein pattern of newly emerged workers, collected immediately before the bees were confined or returned to the colony. This pattern changed as the bees maintained in the colony aged (Fig. 2, lane 3). However, the changes depended on the social environment as shown by the protein pattern of confined workers (Fig. 2, lane 4).

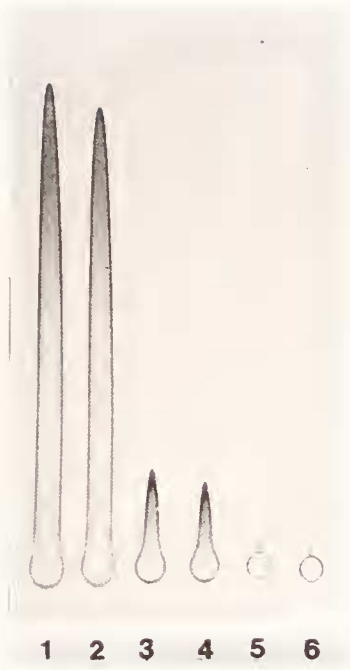

Fig. 1. Rocket immunoelectrophoresis of worker haemolymph. 150 µl queen egg antiserum in 15 ml agarose gel. Antigen: 2 µl of a haemolymph pool per well. Staining: Coomassie Brilliant Blue R-250. Adult workers (6 days) maintained naturally in the colony (1, 2), confined for 15-16 days (3, 4) and fed beebread and candy and confined for 6-days (5, 6) and fed the same diet.

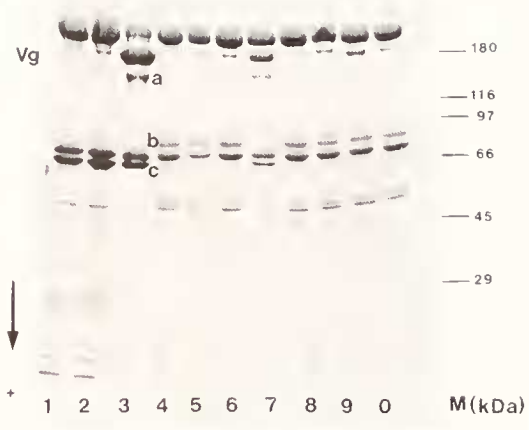

Fig. 2. SDS-PAGE (5.0–12%). Coomassie Blue staining. Patterns of worker haemolymph proteins. (1, 2) Newly emerged workers. Haemolymph diluted 1: 2 (v/v) in sample buffer; (3, 7) 6-day old workers maintained naturally in the colony. Haemolymph diluted (3) 1: 20 (v/v) and (7) 1: 40 (v/v) in sample buffer. Workers confined for 6 days after emergence and treated with PPN (10), JHI (5,9), JHIII (6), or acetone (8, 11), or untreated (4). Haemolymph diluted 1: 2 (v/v) in sample buffer. (M) - molecular weights in kDa according to marker proteins. Note: Columns should be numbered 1-11.

The haemolymph pattern of workers confined for 15-16 days and fed candy and pollen (Fig. 3, lanes 5 and 6) did not differ from that obtained after a short confinement (Fig. 2, lane 4). However, a discrete increase was observed in the Vg band of workers confined for 15-16 days. The value of diet protein content for protein synthesis is clearly shown in Fig. 3, lanes 3, 4, corresponding to the protein pattern of a haemolymph pool from workers maintained on a carbohydrate (syrup) diet for 15-16 days. In the same figure, the protein pattern of haemolymph from confined workers can also be compared with that of newly emerged ones (lanes 7, 8) and with that of workers maintained in the colony for 15 days (lanes 1, 2).

There is no Vg in workers confined for 15-16 days with a queen

Workers confined for 15-16 days in the absence of a queen had Vg in their haemolymph when fed on the beebread and candy mixture, as can be seen by immunoelectrophoresis Fig. 1, wells 3 and 4. However if a queen is confined together with a group of 150-200 workers during this same time interval, from emergence until 15-16 days, the workers will not have Vg in the haemolymph. Thus, the queen effect on Vg synthesis could be observed even in a different environment condition, i.e. that established by confinement. The haemolymph protein SDS-PAGE pattern of these workers was similar to that of bees confined in the absence of a queen, except for a weak Vg band present in the latter (results not shown).

PPN induces Vg titre increase in workers confined for 6 days

Workers confined for six days after emer-

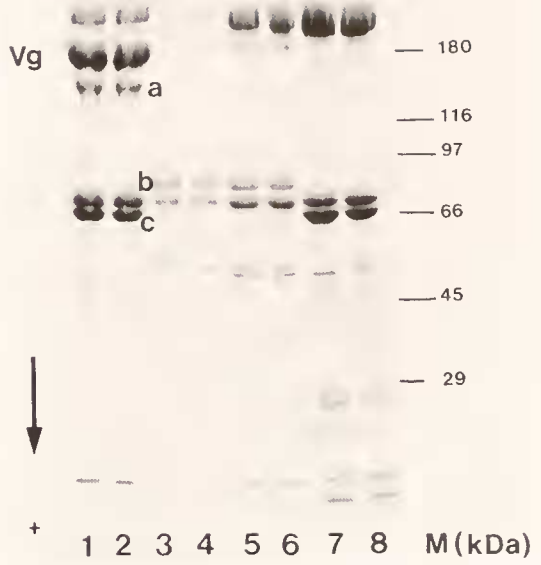

Fig. 3. SDS-PAGE (5.0-12%), Coomassie Blue staining. Patterns of worker haemolymph proteins. (1, 2) 15–day old workers maintained naturally in the colony. Haemolymph diluted 1: 20 (v/v) in sample buffer; (3, 4) workers confined for 15-16 days after emergence and fed syrup or (5, 6) beebread and candy; haemolymph diluted 1: 2 (v/v) in sample buffer; (7, 8) Newly emerged workers. Haemolymph diluted 1: 2 (v/v) in sample buffer. (M) - molecular weights in kDa according to marker proteins.

gence, fed beebread and candy and treated with PPN consistently showed a significant increase ($p<0.001$) in haemolymph Vg concentration (Fig. 4, wells 3, 4, and Fig. 5, wells 3, 4) when compared to confined workers treated with acetone (Fig. 4 wells 1, 2, 7, 8; Fig. 5 well 1) or untreated (Fig. 4, wells 9, 10). This increase corresponded to approximately 17% of the Vg levels in the haemolymph of workers naturally maintained in the colonies (Fig. 4 wells 11, 12; Fig. 5, well 5). To calculate Vg percentage, we used a total of 9 haemolymph pools derived from 9 experiments, each consisting of 150-200 workers treated with PPN. In this case, Vg concentration in control a (workers maintained in the colonies) was considered to be 100% (Table II). Table II also shows that the percentage of Vg detected in acetone-treated workers (control b, 7.4 + 3.2%) did not differ ($0.5>p>0.4$) from that detected in the untreated control c (6.0 ± 2.1%).

The Vg titre detected in workers treated with PPN was similar to that detected in workers confined for 15-16 days and feeding on pollen and candy (Fig. 1, wells 3 and 4). This Vg concentration, however, was never detected in controls c (workers confined for six days and feeding on pollen and candy) or b (workers confined for six days, feeding on pollen and candy and treated

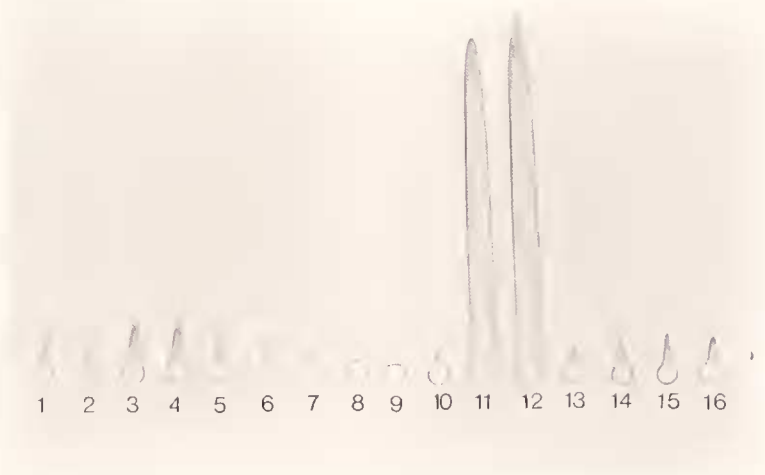

Fig. 4. Rocket immunoelectrophoresis of worker haemolymph. 150 µl queen egg antiserum in 15 ml agarose gel. Antigen: 2 µl of a haemolymph pool per well. Staining: Coomassie Brilliant Blue R-250. Adult workers confined for 6 days after emergence and treated with JHIII (13, 14), JHI (5, 6, 15. 16), PPN (3, 4), or acetone (control b, 1, 2, 7, 8), or untreated (control c, 9, 10). These confined workers were fed beebread and candy. Wells 11 and 12 correspond to the Vg of adult 6-day old workers maintained naturally in colonies.

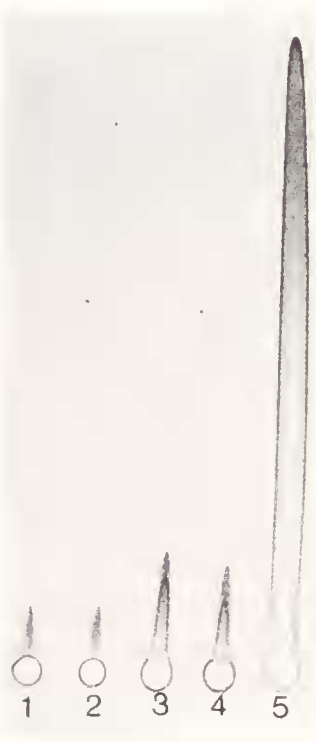

Fig. 5. Rocket immunoelectrophoresis of worker haemolymph 150 µl queen egg antiserum in 15 ml agarose gel. Antigen: 2 µl of a haemolymph pool per well. Staining: Coomassie Brilliant Blue R-250. Adult workers confined for 6 days after emergence, fed beebread and candy and treated with acetone (control b, well 1), JHI (well 2), or PPN (wells 3 and 4). Well 5 corresponds to the Vg of adult 6-day old workers maintained naturally in colonies.

with acetone). Thus, under conditions of prolonged confinement, there is an increase in Vg concentration in haemolymph during the second week of confinement, but this increase never occurs during the first week of confinement unless the workers are treated with PPN.

PPN does not change the SDS-PAGE protein pattern

The protein pattern (Fig. 2) from workers confined for six days and treated with PPN (lane 10) was similar to that observed in the acetone-treated (lanes 8, 11) and untreated controls (lane 4), except by an increase in Vg band.

PPN does not significantly change the total protein concentration in the haemolymph

There was no significant difference in haemolymph protein content between PPN-treated workers and their controls: acetone treated (control b, $p>0.35$) and untreated (control c, $p>0.15$). (Table III).

Comparison between untreated groups (control c) and the groups maintained in the colonies (control a) demonstrated a drastic decrease in haemolymph protein concentration in the first group, maintained under confinement conditions, even when beebread and candy was supplied.

JHI and JHIII did not induce Vg titre increase in workers confined for 6 days

Treatment with JHI (Fig. 4, wells 5, 6, 15, 16 and Fig. 5, well 2) or JHIII (Fig. 4, wells 13, 14) under the same experimental conditions as those with PPN did not increase Vg concentration in haemolymph. The Vg peaks detected in the workers treated with JHI or JHIII did not differ statistically ($p>0.15$ and $p>0.5$, respectively) from the acetone-treated control b (Fig. 4, wells 1, 2, 7, 8 and Fig. 5, well 1 as showed in Table IV). However, the Vg peaks obtained from workers treated with JHI, a hormone not synthesized by *Apis mellifera* (Robinson et al. 1987), were found to be slightly higher than the peaks obtained for workers treated with acetone or with JHIII, the natural hormone of these bees. This result should be considered with caution since it is based only on the data obtained for two haemolymph pools from workers treated with JHI or JHIII. But we do not exclude the possibility of this homologue (JHI) being more effective on *Apis mellifera* than JHIII.

JHI and JHIII did not change the SDS-PAGE protein pattern

The haemolymph protein pattern of workers confined for six days and treated with JHI (Fig. 2, lanes 5, 9) or JHIII (Fig. 2, lane 6) was similar to that observed in the controls treated with acetone (Fig. 2, lanes 8, 11) or untreated (Fig. 2, lane 4).

DISCUSSION

Effect of confinement, queen presence and diet on haemolymph protein composition

Our results show that the normal Vg titre in workers mainly depends on social environment established in the colony. When workers were removed from the colony, and maintained during 6 days in an appropriate environment (where mortality was practically zero) and on a protein-rich diet, profound physiological changes occurred, that inhibited Vg titre and probably Vg synthesis. The initiation of Vg synthesis in these workers can be observed if confinement is lenghthened for 15-16 days. But the haemolymph Vg peak detected by immunoelectrophoresis in these workers (after 15-16 days of confinement) is smaller than that observed in colony reared workers. The onset of Vg in the haemolymph of 15-16 days confined workers is dependent on administration of a protein-rich diet: Vg is not detected in these confined workers fed a diet without protein such as syrup, or a low protein content diet, such as candy made with honey. Besides if a queen is confined with the workers for 15-16 days no Vg is produced although a protein rich diet (beebread and pollen) had been available.

The influence of factors related to the social environment, on Vg synthesis has been studied in social Hymenoptera, especially the interaction among individuals of different castes in the colonies. Reproductively active queens characteristically inhibit Vg synthesis and egg-laying in other females capable of reproduction. This fact has been well documented in *A. mellifera* (Engels et al. 1990), *Melipona* (Engels and Imperatriz-Fonseca 1990), and *Camponotus festinatus* (Martinez and Wheeler 1991) among other social insects. We verify that *A. mellifera* queens can inhibit Vg synthesis in workers even under conditions of confinement, i.e., far from the normal colony environment. Engels et al. (1990) maintained groups of 25 workers for three weeks confined from emergence on a piece of comb, in the presence and absence of a queen. They detected that both worker groups had Vg in the haemolymph, but the Vg titre of workers confined in the absence of a queen was higher than that of workers confined in the presence of a queen. In our experiments, we found that workers confined without a queen for 15-16 days produced Vg when fed beebread and candy. However, when the workers were confined with a queen during this same period of time, no Vg was detected in the haemolymph. Perhaps, the experimental conditions employed by Engels *et al.* 1990, with a piece of comb (with brood?) within the confinement cage, provided a more favorable environment (maybe more similar to that of a normal colony) that permitted Vg synthesis even in the presence of a queen. For comparison, other conditions employed in both experiments should also be taken into account, such as the age of the queens used, the size of the worker population and the time of confinement.

Confinement affects not only Vg synthesis, which is also controlled by the queen and by the food available, but also the synthesis of other haemolymph proteins, as determined by SDS-PAGE. The polypeptide a showed marked reduction, the polypeptide c was not detected, whereas another, b, showed markedly increased titer (Figs. 2 and 3). This indicates that confinement can simultaneously provoke contrasting gene expressions. This was observed in confined workers fed proteic or non-proteic diets. Workers maintained on a syrup diet had a lower protein content, but similar protein pattern in the haemolymph (Fig. 3) when compared to workers maintained on a proteic diet. Thus the social interactions and not the protein supply are responsible for the contrasting gene expressions mentioned above.

Action of PPN, JHIII and JHI on haemolymph protein composition changed by confinement

A significant ($p<0.001$) increase in Vg titre ocurred in workers with confinement-induced Vg deficiency, after treatment with PPN. However the observed induction was partial. Apparently other factors are also involved in the regulation of normal Vg synthesis. The action of these factors may be prevented or impaired by the confinement conditions employed.

PPN seems to be specific for Vg synthesis, since the haemolymph SDS-PAGE pattern of the other proteins in confined workers apparently did not change when treated with this JH analogue. However as the small increase in Vg titre induced by PPN was not reflected in total protein measurement we can not exclude the possibility that PPN also partially inhibits other haemolymph

protein(s).

Contrary to what is observed with PPN, the natural JHIII (Robinson *et al.* 1987) and its homologue JHI did not significantly increase Vg concentration in haemolymph. JHIII and JHI also did not seem to modify the haemolymph protein pattern induced by confinement. We should consider that PPN may have caused an increase in Vg titre in haemolymph by being a more potent analogue. PPN is considered to be one of the most effective juvenile hormone analogues known for locusts (De Kort and Koopmanschap 1991), with a strong juvenilizing effect on these insects. PPN has a strong morphogenetic effect when topically applied to *A. mellifera* larvae. Larvae treated with 1 µg undergo metamorphosis, but the pupae show drastic changes in pigmentation, especially in the eyes and thorax, and can also die before emergence (Bitondi et al., unpublished data). Similarly to what occurs in *Locusta*, the effect of PPN on *A. mellifera* larvae and pupae is more drastic when compared to the effects induced by JHIII or JHI.

Kaatz (1985) proposed a model of regulation of Vg synthesis in *A. mellifera* queens. According to this model, Vg synthesis may be influenced by JH but also by ecdysteroids and by a haemolymph factor. These factors mentioned by Kaatz (1985) may be involved in the regulation of normal Vg synthesis in workers. However, the simple adaptation to workers of the model proposed for queens should be considered with caution since some data obtained in studies on Vg synthesis regulation in queens indicate that this regulation may differ between the two castes (Engels *et al.* 1990).

In summary the absence of one or more types of social interaction induced by confinement caused a reduction of total protein content in haemolymph, induction and repression of protein synthesis and impairment of Vg synthesis in *A. mellifera* workers. Only haemolymph Vg titer can be partially recovered by PPN treatment of confined workers.

ACKNOWLEDGMENTS

This work was supported by grants from the Fundação de Amparo á Pesquisa do Estado de São Paulo (FAPESP) Proc. 91/2587-8.

We thank Dr. H. Oouchi, Sumitomo Chemical Co., Ltd, Osaka, Japan, for providing the pyriproxyfen, and P.R. Epifânio and L. R. Aguiar for the technical assistance. We thank also Dr. David De Jong for correcting the English.

LITERATURE CITED

Adams, T. S. and P. A Filipi. 1988. Interaction between juvenile hormone, 20-hydroxyecdysone, the *corpus - cardiacum - allatum* complex, and the ovaries in regulating vitellogenin levels in the housefly, *Musca domestica*. *Journal of Insect Physiology* 34: 11-19.

Adams, T. S., H. H. Hagedorn and G. D. Wheelock. 1985. Haemolymph ecdysteroid in the housefly, *Musca domestica*, during oögenesis and its relationship with vitellogenin levels. *Journal of Insect Physiology* 31: 91-97.

Agui, N., S. Izumi and S. Tomino. 1985. The role of ecdysteroids and juvenoids in vitellogenin levels and follicle development in the housefly, *Musca domestica*. *Applied Entomology and Zoology* 20: 179-188.

Agui, N., T. Shimada, S. Izumi and S. Tomino. 1991. Hormonal control of vitellogenin mRNA levels in the male and female housefly, *Musca domestica*. *Journal of Insect Physiology* 37: 383-390.

Bianchi, A. G. de and S. D. Pereira. 1987. Time of synthesis of *Musca domestica* vitellogenin during the first gonotrophic cycle. *Comparative Biochemistry, and Physiology* 86B(4): 697-700.

Borovsky, D., B. R. Thomas, D. A. Carlson, L. R. Whisenton and M. S. Fuchs. 1985. Juvenile hormone and 20-hydroxyecdysone as primary and secondary stimuli of vitellogenesis in *Aedes aegypti*. *Archives of Insect Biochemistry and Physiology* 2: 75-90.

Bownes, M.. 1989. The roles of juvenile hormone, ecdysone and the ovary in the control of *Drosophila* vitellogenesis. *Journal of Insect Physiology* 35(5): 409-413.

Bownes, M. and G. Reid. 1990. The role of the ovary and nutritional signals in the regulation of fat body yolk protein gene expression in *Drosophila melanogaster*. *Journal of Insect Physiology* 36(7): 471-479.

Bradford, M.. 1976. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein dye binding. *Analytical Biochemistry* 72: 248-254.

Davis, R. E., T. J. Kelly, E. P. Masler, H. W. Fescemyer, B. S. Thyagaraja and A. B. Borkovec. 1990. Hormonal control of vitellogenesis in the gypsy moth, *Lymantria dispar* (L.): suppression of haemolymph vitellogenin by the juvenile hormone analogue, methoprene. *Journal of Insect Physiology* 36(4): 231-238.

De Kort, C. A. D. and A. B. Koopmanschap. 1991. A juvenile hormone analogue affects the protein pattern of the haemolymph in last-instar larvae of *Locusta migratoria*. *Journal of Insect Physiology* 37(2): 87-93.

Don-Wheeler, G. and F. Engelmann. 1991. The female - and male - produced vitellogenins of *Leucophaea maderae*. *Journal of Insect Physiology* 37(12): 869-882.

Elliot, R. H. and C. Gillot. 1978. The neuro-endocrine control of protein metabolism in the migratory grasshopper, *Melanoplus sanguinipes*. *Journal of Insect Physiology* 24: 119-126.

Engels, W. 1974. Occurrence and significance of vitellogenins in female castes of social Hymenoptera. *American Zoologists* 14: 1229-1237.

Engels, W. and H. Fahrenhorst. 1974. Alters - und kasten - spezifische Veränderungen der Haemolymph - Protein - Spektren bei *Apis mellifica*. *Wilhelm Roux' Archiv* 174: 285-296.

Engels, W. and V. L. Imperatriz-Fonseca. 1990. Caste development, reproductive strategies, and control of fertility in honey bees and stingless bees. *In*: Engels, W. (ed.) *Social Insects. An evolutionary approach to castes and reproduction*. Springer-Verlag, Berlin, pp167-230.

Engels, W., H. Kaatz, A. Zillikens, Z. L. Paulino-Simões, A. Trube, R. Braun and F. Dittrich. 1990. Honey bee reproduction: vitellogenin and caste-specific regulation of fertility. *In*: M. Hoshi and O. Yamashita (eds.) *Advances in invertebrate reproduction* 5, 495-502. Elsevier, Amsterdam.

Engels, W and P. S. Ramamurty. 1976. Effects of carbon dioxide on vitellogenin metabolism in unmated queen honeybees. *Journal of Apiculture Research* 15: 3-10.

Fluri, P., H. Wille; L. Gerig and M. Lüscher. 1977. Juvenile hormone, vitellogenin and haemocyte composition in winter worker honey bees (*Apis mellifera*). *Experientia* 33: 1240-1241.

Girardie, J., O. Richard and A. Girardie. 1992. Time-dependent variations in the activity of a novel ovary maturating neurohormone from the nervous *corpora cardiaca* during oögenesis in the locust, *Locusta migratoria migratorioides*. *Journal of Insect Physiology* 38: 215-221.

Hagedorn, H. H.. 1985. The role of ecdysteroids in reproduction. In *Comprehensive Insect Physiology, Biochemistry and Pharmacology*. (Edited by G. A. Kerkut and L. I. Gilbert), vol. 8, pp 205-262. Pergamon Press, Oxford.

Harnish, D. G. and B. N. White. 1982. Insect vitellins: identification, purification, and characterization from eight orders. *Journal of Experimental Zoology* 320: 1-10.

Hatakeyama, M. e K. Oishi. 1990. Induction of vitellogenin synthesis and maturation of transplanted previtellogenic eggs by juvenile hormone III in males of the sawfly, *Athalia rosae*. *Journal of Insect Physiology* 36: 791-797.

Huang, Z. Y., G. E. Robinson, S. S. Tobe, K. J. Yagi, C. Strambi, A. Strambi and B. Stay. 1991. Hormonal regulation of behavioral development in the honey bee is based on changes in the rate of juvenile hormone biosynthesis. *Journal of Insect Physiology* 37(10): 733-741.

Kaatz, H. H.. 1985. Initiation und Regulation der Vitellogenin-Synthese bei der Bienenkönigin (*Apis mellifera* L.). Inaugural-Dissertation, Universität Tübingen, pp. 146-159.

Keeley, L. L. and S. C. McKercher. 1985. Endocrine regulations of ovarian maturation in the cockroach *Blaberus discoidales*. *Comparative Biochemistry and Physiology* 80A: 115-121.

Keeley, L. L., S. M. Sowa, T. K. Hayes and J. Y. Bradfield. 1988. Neuroendocrine and juvenile hormone effects on fat body protein synthesis during the reproductive cycle in female *Blaberus discoidalis* cockroaches. *General Comparative Endocrinology* 72: 364-373.

Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T_4. *Nature* 227: 680-685.

Ma, M., J. -Z. Zhang and H. Gong. 1988. Permissive action of juvenile hormone on vitellogenin production by the mosquito *Aedes aegypti*. *Journal of Insect Physiology* 34: 593-596.

Martinez, T. and D. Wheeler. 1991. Effect of the queen, brood and worker caste on haemolymph vitellogenin titre in *Camponotus festinatus* workers. *Journal of Insect Physiology* 37(5): 347-352.

Robinson, G. E., Strambi, A., Strambi, C., Paulino-Simões, Z.L., Tozeto, S.O., Barbosa, J. M. N. 1987. Juvenile hormone titers in Africanized and European honey bees in Brazil. *Gen. Comp. Endocrinol.* 66: 457-459.

Röseler, P. F. and I. Röseler. 1988. Influence of juvenile hormone on fat body metabolism in ovariolectomized queens of the bumblebee, *Bombus terrestris*. *Insect Biochemistry* 18(6): 557-563.

Rutz, W., L. Gerig;, H. Wille and M. Luscher. 1976. The function of juvenile hormone in adult worker honeybees, *Apis mellifera*. *Journal of Insect Physiology* 22: 1485-1491.

Schwartz, M. B., T. J. Kelly, R. B. Imberski and E. C. Rubenstein. 1985. The effects of nutrition and methoprene treatment on ovarian ecdysteroid synthesis in *Drosophila melanogaster*. *Journal of Insect Physiology* 31: 947-957.

Shapiro, J. P., J. H. Law and M. A. Wells. 1988. Lipid transport in insects. *Annual Review of Entomology* 33: 297-318.

Simões, Z. L. P. 1980. Estudo da vitelogenina e da vitelina em *Apis mellifera* L. (Hymenoptera : Apoidea). Tese de doutoramento, Universidade de São Paulo. pp.: 1-108.

Socha, R., J. Sula, D. Kodrík and I. Gelbic. 1991. Hormonal control of vitellogenin synthesis in *Pyrrhocoris apterus* (L.) (Heteroptera). *Journal of Insect Physiology* 37( ): 805-816.

Trenczek, T. and W. Engels. 1986. Occurrence of vitellogenin in drone honeybees. *International Journal of Invertebrate Reprodution and Development* 10: 307-311.

Trenczek, T., A. Zillikens and W. Engels. 1989. Developmental patterns of vitellogenin haemolymph titre and rate of synthesis in adult drone honey bees (*Apis mellifera*). *Journal of Insect Physiology* 35: 475-481.

Wheeler, D. E. and J. K. Kawooya. 1990. Purification and characterization of honey bee vitellogenin. *Archives of Insect Biochemistry and Physiology* 14: 253-267.

Wojchowski, D. M. and J. G. Kunkel. 1987. Purification of two distinct oocyte vitellins and identification of their corresponding vitellogenins in fat body and hemolymph of *Blaberus discoidalis*. *Insect Biochemistry* 17: 189-198.

Wyatt, G. R.. 1988. Vitellogenin synthesis and the analysis of juvenile hormone action in locust fat body. *Canadian Journal of Zoology* 66: 2600-2610.

Yin, C. -M., B. -X. Zou, S. -X. Yi and J. G. Jr. Stoffolano. 1990. Ecdysteroid activity during oögenesis in the black blowfly, *Phormia regina* (Meigen). *Journal of Insect Physiology* 36: 375-382.

Table I. Haemolymph pools prepared from workers confined for 6 or 15-16 days in the presence or absence of a queen, fed on different diets and submitted or not to hormonal treatment.

No. of pools analysed	Confinement (days from emergence)	Diet	Hormonal or acetone treatment	Queen
1	6	Syrup	-	absent
1	6	Candy	-	absent
5	6	breebread and candy	-	absent
2	6	breebread and candy	JHIII	absent
2	6	breebread and candy	JHI	absent
9	6	breebread and candy	PPN	absent
6	6	breebread and candy	Acetone	absent
2	15-16	Syrup	-	absent
1	15-16	Candy	-	absent
3	15-16	breebread and candy	-	absent
3	15-16	breebread and candy	-	present

Table II - Vitellogenin (Vg) in haemolymph pools from confined workers treated with PPN or acetone (control b), from untreated confined workers (control c) and from workers maintained in colonies (control a).

	PPN treated		Acetone treated (control b)		Untreated (control c)		Maintained in the colonies (control a)	
Experiment No.	Peak Height (mm)	Vg# (%)	Peak Height (mm)	Vg# (%)	Peak Height (mm)	Vg# (%)	Peak Height (mm)	Vg# (%)
1	5.0	16.13	3.5	11.29	1.5	4.39	31.0	100
2	5.5	17.74	*	*	*	*	*	*
3	4.0	12.90	*	*	*	*	*	*
4	6.5	20.97	*	*	*	*	*	*
5	6.5	15.12	2.0	4 65	3.0	6.98	43.0	100
6	7.0	17.95	1.0	2.56	3.0	7.69	39.0	100
7	8.5	21.80	3.5	8.97	3.0	7.69	+	+
8	4.5	14.52	2.5	8.06	1.0	3.22	31.0	100
9	6.5	14.61	4.0	8 99	z	z	44 5	100
MEAN ± SD		16.9±3.0		7.4±3.2		6.0±2.1		100

\# Percent Vg in relation to workers maintained in the colony (control a = 100% Vg);
* Since experiments 1-4 were performed simultaneously, the same controls (a, b and c) were used in each;
z There was no control c for experiment 9;
\+ Experiments 6 and 7 were performed during subsequent weeks. The same control a was used.

Table III. Protein titre* (µg/µl haemolymph in BSA equivalents) of 6-day old workers confined from emergence and treated with PPN or acetone (control b), untreated (control c) and maintained in the colony (control a).

Experiment No.	PPN treated µg/µl	Acetone treated (control b) µg/µl	Untreated (control c) µg/µl	Maintained in the colonies (control a) µg/µl
1	6.76	7.22	5.04	53.15
2	7.03	—	—	—
3	6.81	—	—	—
4	8.38	—	—	—
5	—	—	—	33.88
6	7.0	6.62	5.64	31.05
7	7.98	6.89	7.93	—
MEAN ± SD	7.3±0.7	6.9±0.3	6.2±1.5	39.4±12.0

(*)Protein concentrations were only measured in 6 of the 9 experimental groups treated with PPN, in 3 of the 6 groups treated with acetone, in 3 of the 5 untreated groups, and in 3 of the 5 groups maintained in the colonies.

Table IV. Vitellogenin (Vg) percent in haemolymph pools from confined workers treated with JHIII, JHI or acetone (control b) and from workers maintained in the colonies (control a).

Experiment No.	JHI treated		JHIII treated		Acetone treated		Maintained in the colonies	
	Peak Height (mm)	Vg (%)	Peak Height (mm)	Vg (%)	Peak Height (mm)	Vg (%)	Peak Height (mm)	Vg (%)
1	4.5	10.00			4.0	8.89	*	*
2	4.0	8.89	3.5	7.78	1.5	3.33	45	100
3			2.0	4.60	2.0	4.60	43.5	100
MEAN ± SD		9.4±0.8		6.2±2.2		5.6±2.9		

* Since experiments 1 and 2 were performed on subsequent days, the same control a was used.